Table S1. Examples of TLR2-inducible genes inhibited by IFN-γ

Gene symbol*	Affymetrix #	Fold suppression by IFN-γ**	Sensitivity to CHX***
HEY1	218839_at	127.4	–
SLAMF1	206181_at	65.0	↓↓
TFPI2	209278_s_at	34.0	↓
CXCL5	205101_s_at	28.4	↓
SOCS2	203372_s_at	24.6	–
MMP1	204475_at	19.3	↓↓
SERPINB2	204614_at	19.2	↓↓
CRLF2	208303_at	13.9	–
DLL1	224215_s_at	12.1	↓
PTX3	206157_at	12.1	↓↓
IL10	207433_at	11.8	↓↓
IL7R	205798_at	9.5	–
CCL18	209924_at	8.3	–
ITGB3	204627_s_at	8.1	↓↓
IL24	206569_at	7.9	–
TNIP3	206655_at	6.9	↓↓
CXCL6	206336_at	4.9	↓↓
CCL24	221463_at	4.6	↓↓
BATF	205965_at	4.1	↓↓
IL2RA	206341_at	3.9	–
CCL15	210390_s_at	3.1	↓↓
HES1	203394_s_at	3.1	–
MYC	202431_s_at	2.2	–

Regulation of all genes shown was confirmed by real time PCR in at least 2 independent donors.

MIAME-compliant complete data set was deposited to Gene Expression Omnibus (GEO) database with the accession number GSE11864.

* Genes encoding Notch pathway components or Notch target genes are highlighted in yellow.
** Average fold change of two independent microarray experiments.
*** Assessed by real time PCR.
(↓↓: >80% inhibition; ↓: >50%-80 inhibition; -: <50% inhibition)

Table S2. Inhibitor profile of a subset of IFN-γ-suppressed, CHX-resistant genes*

Gene symbol	Effects of inhibitors**		
	γ-secretase inhibitors	Bay11-7082	MAPK inhibitors
AREG	ND	ND	↓↓
CCL18	ND	ND	↓↓
CRLF2	ND	ND	↓↓
HES1	↓	↓↓	↓↓
HEY1	↓↓	↓	–
IL24	ND	ND	↓↓
IL2RA	↓↓	↓↓	↓↓
IL7R	↓↓	ND	↓
MYC	ND	ND	–
SOCS2	ND	ND	↓

* Data were summarized from two to four independent experiments.
** Assessed by real time PCR.
↓↓: >80% inhibition; ↓: >50%-80 inhibition; –: <50% inhibition; ND: not determined

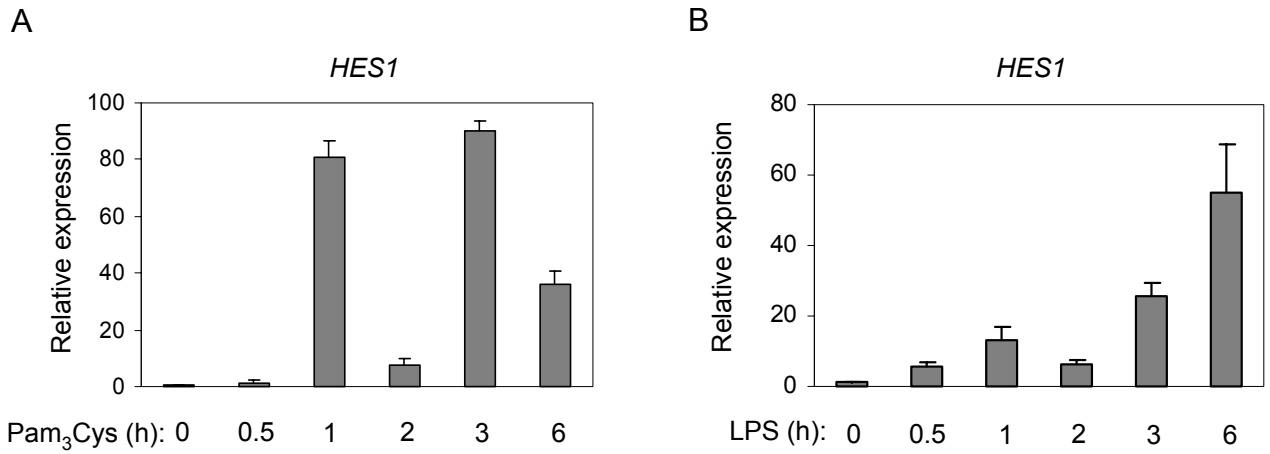

Figure S1. Oscillatory pattern of TLR-induced *HES1* expression

Human primary macrophages were stimulated with 100 ng/ml of Pam3Cys (A) or 1 ng/ml of LPS (B) for the indicated time periods. mRNA levels of Hes1 were measured by real time PCR. Representative experiments in which oscillations of Hes1 mRNA were detected are shown.

Figure S2. NICD levels in macrophages activated with LPS.

(A) Human primary macrophages were stimulated with 10 ng/ml of LPS for the indicated time periods. Whole cell extracts were subjected to western blotting using an NICD1-specific antibody that does not recognize full length Notch1 protein (upper panel). The molecular weight of NICD1 is 110kD. A duplicate filter was blotted with an anti-p38 antibody (lower panel). Data are representative of three independent experiments.

(B) Human primary macrophages were stimulated with 10 ng/ml of LPS for the indicated time periods. Whole cell extracts were subjected to western blotting using an antibody against Notch2. Upper panel shows the cleaved NICD2 with the molecular weight of 110kD. The same filter was blotted with an anti-SHP2 antibody (lower panel).

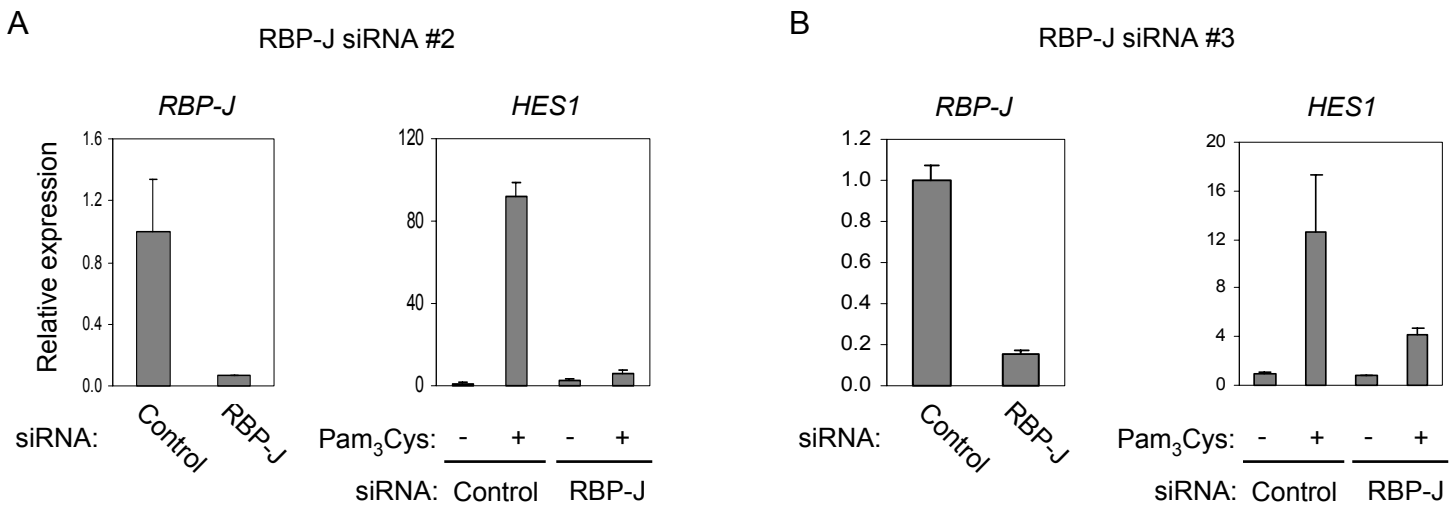

Figure S3. Dependency of Hes1 induction on RBP-J is confirmed by two additional independent siRNAs.

To confirm the results in Figure 3A, primary human macrophages were transfected with control non-targeting siRNA or two different RBP-J-specific siRNAs obtained from Invitrogen. 4 days post transfection, cells were stimulated with Pam_3Cys (10 ng/ml) for 3 h, and mRNA was measured using real time PCR.

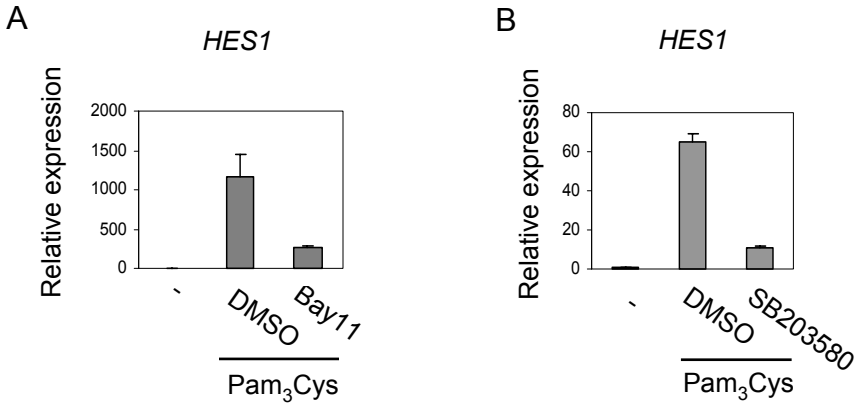

Figure S4. TLR2 induction of *HES1* is dependent on p38 and IKKs

Human macrophages were preincubated with 10 μM of Bay11-7082 (A) or 10 μM of SB203580 (B) for 30 min and stimulated with 10 ng/ml of Pam3Cys for 3 h. mRNA was measured using real time PCR. Results are representative of four independent experiments.

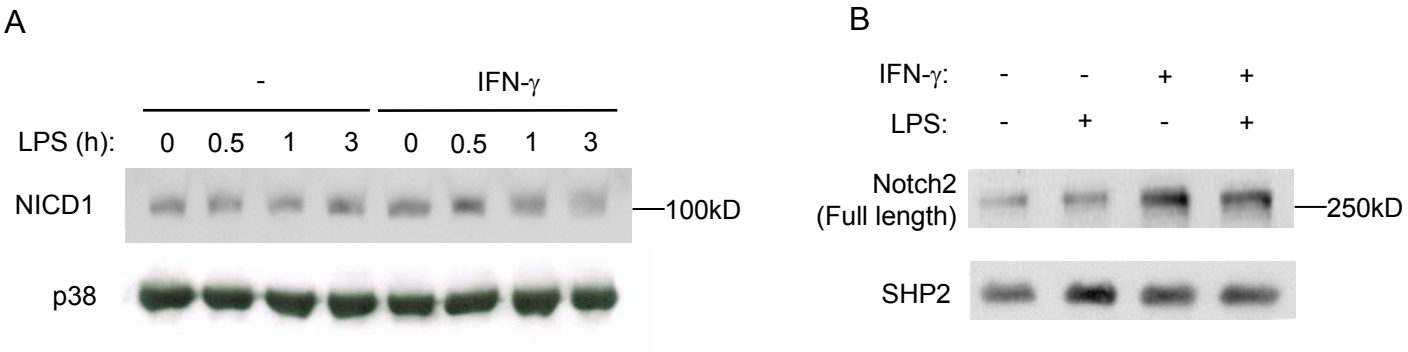

Figure S5. Notch protein levels in macrophages treated with IFN-γ.

(A) Control or IFN-γ primed human primary macrophages were stimulated with 10 ng/ml of LPS for the indicated time periods. Whole cell extracts were subjected to western blotting using a NICD1-specific antibody (upper panel). A duplicate filter was blotted with anti-p38 antibody (lower panel). Data are representative of three independent experiments.

(B) Control or IFN-γ primed human primary macrophages were stimulated with 10 ng/ml of LPS for 6 h. Whole cell extracts were subjected to western blotting using an antibody against Notch2. Upper panel shows the full length Notch2 protein with a molecular weight around 270kD. The same filter was blotted with an anti-SHP2 antibody (lower panel).

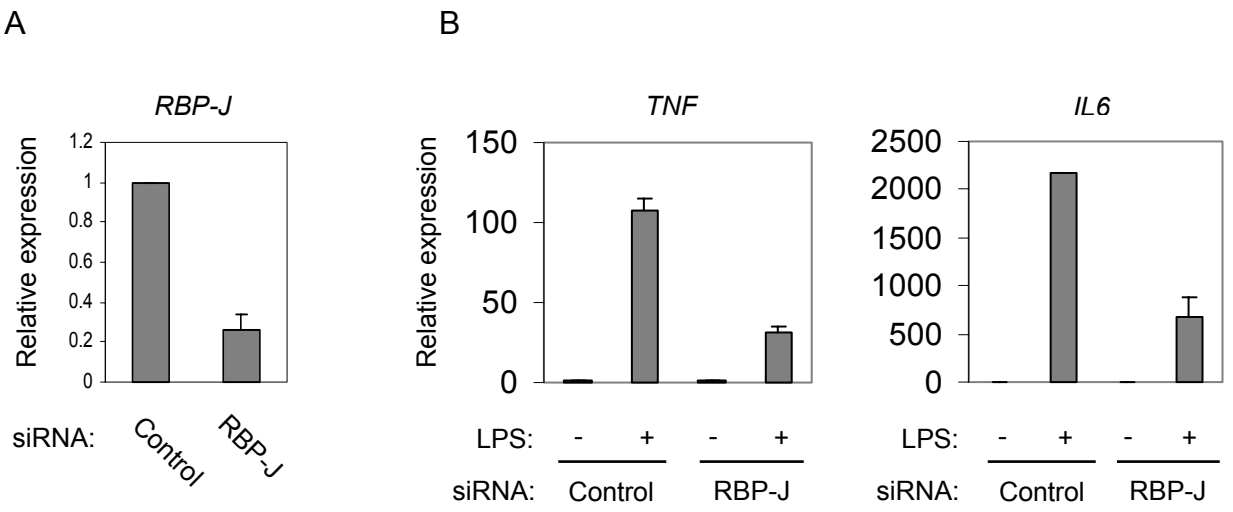

Figure S6. Attenuated expression of TNF and IL-6 in RBP-J-depleted human macrophages.

Primary human macrophages were transfected with control, non-targeting or RBP-J-specific short interfering RNA duplexes obtained from Dharmacon. 4 days post transfection, cells were stimulated with LPS (10 ng/ml) for 3 h, and mRNA was measured using real time PCR. The results are representative of three independent experiments.

(A) RBP-J mRNA expression.

(B) TNF and IL-6 mRNA expression.

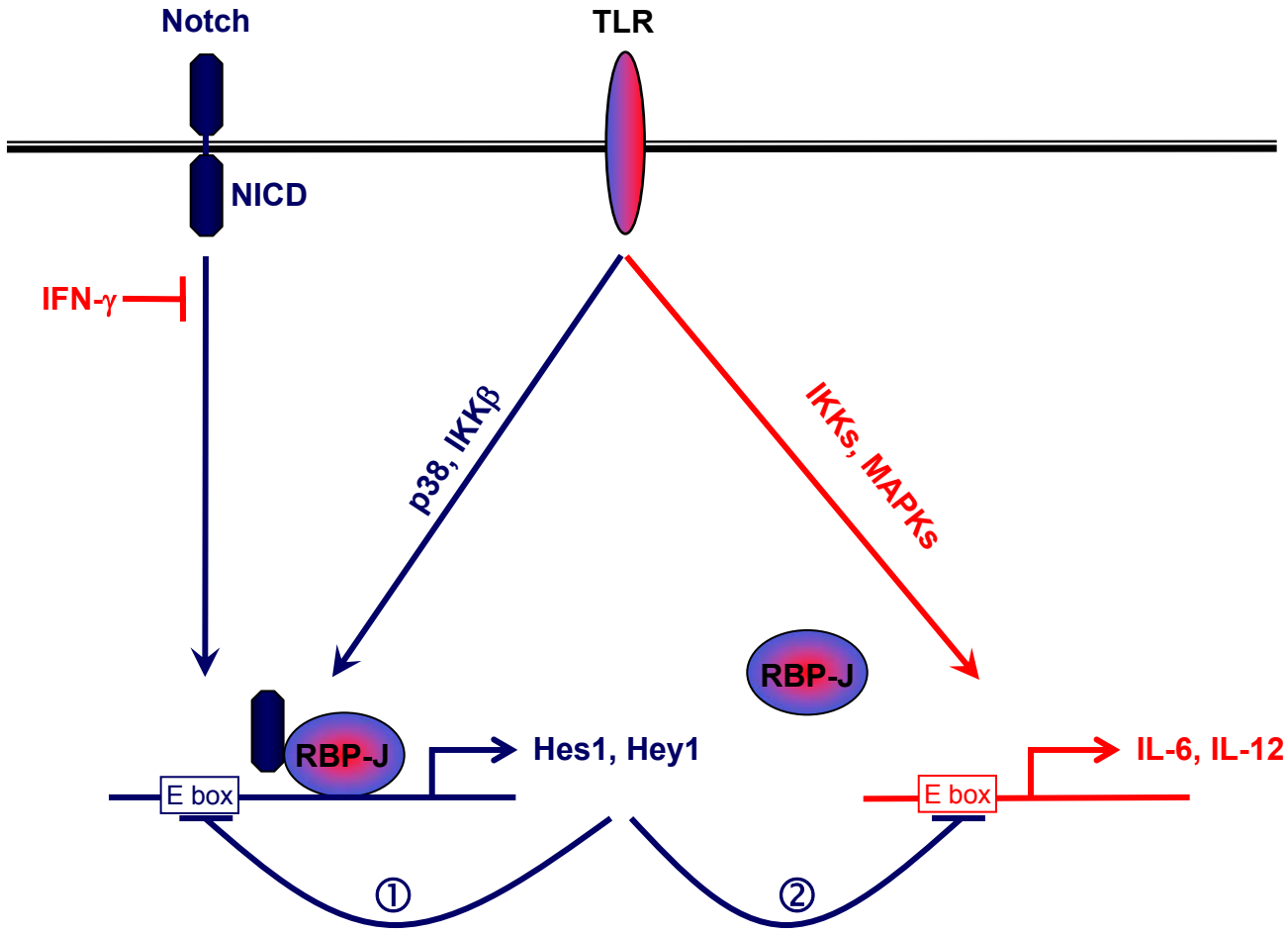

Figure S7. Model for interaction of Notch, TLR, and IFN-γ pathways.

Our findings support a model of cooperation between RBP-J-dependent Notch pathway and IKKβ/p38-dependent TLR pathway in the binary activation of canonical Notch target genes such as Hes1 and Hey1 in macrophages (left, depicted in blue to reflect the anti-inflammatory nature of this pathway). RBP-J also contributes to production of TLR-induced proinflammatory cytokines (right, depicted in red to reflect the pro-inflammatory nature of this pathway). Previous work has established that induced Hes1 and Hey1 feed back to inhibit their own promoters via an E box and thus their own expression (line ①). Hes1 and Hey1 also act *in trans* to suppress TLR induction of cytokines of IL-6/12 family (line ②). Overall, this model introduces the concept of cooperation between Notch and TLR pathways in macrophages with a regulatory circuit that can fine tune macrophage activation. In addition, IFN-γ suppresses Notch signaling and downstream transcription of canonical Notch target genes; inhibition of Hes1 and Hey1 induction may contribute to IFN-g-mediated augmentation of cytokine production.